REMARKS

Applicants have amended Claims 1, 6, 8, and 9. Enabling support for the amendments can be found in the application as filed, and therefore no new matter is contained in the amendments. Reconsideration of the present application and allowance of resulting Claims 1-16, and 35-36 is respectfully requested in view of the amendments and following remarks.

I. Claim Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-16, 35 and 36 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

With regard to Claim 1, the Office Action stated that the phrase "gene" is indefinite. Applicants have amended the foregoing claim to remove the objectionable term in accordance with the suggestion by the Examiner.

With regard to Claim 1(a), the Office Action stated that the term "receptor polynucleotide" is unclear because it is not apparent how a "receptor polynucleotide" differs from a "polynucleotide." Applicants respectfully traverse this rejection. As stated in paragraph 34 of the instant application, one embodiment of the invention comprises a method for the replacement of a DNA of interest, through the use of an irreversible recombinase to replace a receptor polynucleotide with a donor polynucleotide. As such, a "receptor polynucleotide" refers to the DNA of interest that is replaced by a second DNA of interest. It is respectfully submitted that one of ordinary skill in the art, using the specification as a guide, would be able to identify a "receptor polynucleotide" for use in the claimed method. On this basis, it is respectfully submitted that this rejection should be withdrawn.

With regard to Claim 1, the Office Action stated that "two or more of" is unclear. Applicants to amended the foregoing claim to recite "two or more copies of" in accordance with the suggestion by the Examiner.

With regard to Claim 1, the Office Action stated that the recitation of the terms within parentheticals, i.e. (IRS) and (CIRS) is unclear. As stated in paragraph 36 of the instant

specification, the term "irreversible recombination site" is used interchangeably with the term "IRS," and the term "complementary irreversible recombination site" is used interchangeably with the term "CIRS." The terms IRS and CIRS are not meant as additional limitations to the claim. It is respectfully submitted that one of ordinary skill in the art, using the specification as a guide, would understand that the terms "IRS" or a "CIRS" are not additional limitations to the claims. On this basis, it is respectfully submitted that this rejection should be withdrawn.

With regard to Claim 1(b), the Office Action stated that the term "donor polynucleotide" is unclear because it is not apparent how a "donor polynucleotide" differs from a "polynucleotide." Applicants respectfully traverse this rejection. As stated in paragraph 34 of the instant application, one embodiment of the invention comprises a method for the replacement of a DNA of interest, through the use of an irreversible recombinase to replace a receptor polynucleotide with a donor polynucleotide. As such, a "donor polynucleotide" refers to the DNA of interest that replaces a DNA of interest. It is respectfully submitted that one of ordinary skill in the art, using the specification as a guide, would be able to identify a "donor polynucleotide" for use in the claimed method. On this basis, it is respectfully submitted that this rejection should be withdrawn.

With respect to Claim 1(d), the Office Action stated that "the first and second types of recombination sites" lacks antecedent basis, and also stated that recombination occurs between nucleic acids, not between "types" of polynucleotides. Applicants have amended Claim 1 to remove the objectionable term, and to clarify that the recombination is occurring between the nucleic acids of the IRS and the CIRS. On this basis, it is respectfully submitted that this rejection should be withdrawn.

With respect to Claim 12, the Office Action stated that the term "negative selectable marker" is unclear. As stated in paragraph 54 of the instant specification, a target construct, which is to be replaced by a donor polynucleotide, can include a negative selectable marker. As stated therein, "after introduction of the integrating construct and contacting with the recombinase, the cells are then subjected to negative selection to eliminate those cells that retain the negative selectable marker. Suitable examples of negative selection markers are known to those of skill in the art, and include, for example, the Herpes simplex virus thymidine kinase

gene that results in killing the mammalian cells upon contact with ganciclovir." A negative selectable marker therefore is one that allows for the selection of a cell that does not contain the selectable marker, since the presence of the negative selectable marker in a cell confers sensitivity to the selection treatment. On this basis, Applicants submit that one or ordinary skill in the art, using the specification as a guide, would be able to identify a "negative selectable marker" for use in the claimed method. On this basis, it is respectfully submitted that this rejection should be withdrawn.

Pursuant to the rejections, Applicants have amended Claim 1 as shown on the attached sheets, and in accordance with the Examiner's suggestions. Applicants thank the Examiner for her suggestions. Applicants submit that the amendments and remarks overcome the rejections and respectfully request reconsideration and allowance of the amended claims.

II. Rejections under 35 U.S.C. §112

Claims 1-16, 35 and 36 were rejected under 35 U.S.C. §112, first paragraph as being unpatentable since the specification allegedly does not enable any person skilled in the art to make and use the invention as claimed.

The Office Action asserts that the specification does not enable any person skilled in the art to make and use the invention for all eukaryotic cells, all plant cells, all methods of introducing a construct and for all irreversible recombinases. The Office Action argues that all plant cells are not enabled by the specification because of the state of the art and the predictability or lack thereof. The Office Action states on pages 8-9 that "Applicant claims all plant cells. Applicant teaches *Arabidopsis thaliana* plant cells and plants. *Arabidopsis thaliana* is not representative of all plants. Rather *Arabidopsis thaliana* is special in that *Arabidopsis thaliana* has the smallest known plant genome size – (10^7 bp). . . . Plant genomes vary in size from that of Arabidopsis thaliana to larger than 10^{11}. . . *Arabidopsis thaliana* is an excellent model system for plant genetics because of its small genome size and because of *Arabidopsis thaliana*'s lifestyle, short generation time, small plant size, and other well known advantages. Molecular manipulations of plants having larger genome sizes, are more difficult to manipulate because of the sheer physical quantity of DNA. It is unpredictable that all plants would be

equally amenable to chromosomal replacement manipulation as is *Arabidopsis thaliana.* Applicant has provided no guidance on how to predictably eliminate inoperable embodiments from a virtually *ad infini*tum of possibilities other than by random trial and error, which is excessive experimentation and an undue burden." Applicants respectfully traverse this rejection as follows.

In determining whether a patent application is enabled, it must be considered whether a person of ordinary skill in the art could practice the invention without "undue experimentation." *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed Cir. 1998). This judicially-created test sets forth eight *Wands* factors that are to be considered in determining whether a patent application is enabled:

1. the breadth of the claims,
2. the nature of the invention,
3. the state of the prior art,
4. the level of skill possessed by one of ordinary skill in the art,
5. the level of predictability in the art,
6. the amount of direction provided in the application,
7. the existence of working examples in the specification, and
8. the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

While not all of these factors must be considered in making a determination of enablement, the factors must be considered as a group. *Enzo Biochem., Inc. v. Calgene, Inc.*, 188 F.3d 1362, 52 USPQ2d 1129 (Fed. Cir. 1999). Because the *Wands* factors must be considered as a whole, an application that is deficient with respect to one or more factors may still be enabled. For example, providing extensive guidance in the specification and disclosing multiple working examples may compensate for a relatively high level of unpredictability or a low level of skill in the art.

The Office Action states that "in view of the breadth of the claims (any eukaryotic cell, any plant cell, and any irreversible recombinase), the unpredictability of the art, undue trial and error experimentations would be required to enable one skilled in the art to which it pertains,

or with which it is most nearly connected, to make and/or use the invention." Applicants traverse this rejection.

The claims are broad, however, Applicants have provided working examples demonstrating replacement of a chromosomal nucleic acid sequence in yeast using a linear DNA donor construct and a circular DNA donor construct, replacement of a chromosomal nucleic acid sequence in mammalian cells using a circular DNA donor construct, and replacement of a chromosomal nucleic acid sequence in a plant by transferring DNA from a non-homologous chromosome. Applicants have additionally provided prophetic examples for the replacement of DNA in plant cells using linear and concatemeric constructs. The claimed method has been shown in the instant application to be effective in organisms as diverse as yeast, human cells, and plants. Applicant has shown how to make and use the invention in three widely diverse species, and it is therefore submitted that the use of the claimed method to replace a nucleic acid sequence is enabled for eukaryotic cells.

The Office Action asserts that "Arabidopsis thaliana is not representative of all plants. . . . *Arabidopsis thaliana* is an excellent model system for plant genetics because of its small genome size and because of *Arabidopsis thaliana*'s lifestyle, short generation time, small plant size, and other well known advantages. Molecular manipulations of plants having larger genome sizes, are more difficult to manipulate because of the sheer physical quantity of DNA. It is unpredictable that all plants would be equally amenable to chromosomal replacement manipulation as is *Arabidopsis thaliana*."

Applicant respectfully traverses this assertion. Applicant has previously shown that the ϕC31 recombination system can perform site-specific recombination in tobacco, a plant with a genome size some 20 fold higher than *Arabidopsis* (see Examples 4 and 5 in PCT Publication No. WO0107572A2: DNA Recombination in Eukaryotic Cells by the Bacteriophage PhiC31 Recombination System). In addition, Applicant has shown that success with the gene replacement system described and claimed in the instant application with both mouse and human cells. As described above, the initial patent specification described the replacement of a nucleic acid in human cell using a circular substrate. The data on mouse cells has been published in Nature Biotechnology (Belteki, et al., 2003, "Site-specific cassette exchange and germline

transmission with mouse ES cells expressing fC31 integrase," Nature Biotechnology 21: 321-324, attached herewith). The paper describes efficient gene replacement events in mouse cells due to recombination between two IRS in a direct orientation and two CIRS in a direct orientation. This paper further supports that assertion that the claimed methods are successful in eukaryotic cells, and that the methods are enabled for use in eukaryotic cells.

While *Arabidopsis* has a genome size of approximately 100 Mb, human and mouse cells have a genome size of approximately 3,000 Mb. It is notable that both the human and the mouse genome are orders of magnitude larger than the genome of *Arabidopsis*, hence the mere argument that size is a determinant in whether gene replacement can take place with an irreversible site-specific recombination system is erroneous.

In addition, there is not a single documented case where a site-specific recombination system worked in a small genome plant but will not work in a large genome plant. Take for instance, the Cre-lox site-specific recombination system. It was first shown to operate in tobacco. A decade later, Applicant showed efficient site-specific recombination in wheat, a plant with an order of magnitude larger genome than tobacco (Srivastava *et al.*, 1999, "Single-copy transgenic wheat generated through the resolution of complex integration patterns," Proceedings of the National Academy of Sciences USA, 96: 11117-11121, attached herewith). One can speculate that a larger genome size might possibly affect the efficiency of site-specific recombination, due to a possibility that it would take a longer time for the recombinase or integrase to scan the genome for the recombination site, but this was not the case with the Cre-lox system in the wheat genome. The reaction was as efficient in wheat as it was in tobacco. Genome size should not change the specificity of a biochemical reaction. A change in specificity would only be caused by a change in DNA or chromatin structure, but there is no evidence, and nor has the Examiner provided evidence, that different plants have different DNA or chromatin structure.

The above logic also holds true with other DNA enzymes or DNA cis-elements. There has not been a documented case where for instance, a transcription factor works in *Arabidopsis* but does not work in a plant with a larger genome so long as the corresponding promoter was present. Conversely, there has never been a documented case where a promoter

element works in *Arabidopsis*, but does not work in a plant with a larger genome if the appropriate transcription factor was present. The argument in the Office Action that large genomes would prevent a biochemical reaction from operating is purely an opinion, and lacks scientific support.

Applicant concedes that it is generally more difficult to work with plants with a genome size larger than *Arabidopsis*. Conducting experiments on *Arabidopsis* is easier because it is a model system with optimized transformation methods and a rapid generation time. However, for those who are skilled at transforming crop plants, the same experiments can be performed in other plants, it just requires more skill, more time and more tissue culture and greenhouse resources. Take for example, the Cre-lox site-specific recombination system. Prior to 1999, the only documented cases of site-specific recombination were reported in tobacco and *Arabidopsis*. Since 1999, the Cre-lox system has been shown to work in wheat, corn and rice (Srivastava *et al.*, *supra*; Srivastava & Ow, 2001, "Single copy primary transformants of maize obtained through the co-introduction of a recombinase-expressing construct," Plant Molecular Biology, 46:561-566; Srivastava & Ow, 2002, "Biolistic mediated site-specific integration in rice," Molecular Breeding, 8: 345-350, attached herewith). Rice has a genome size of approximately 430 Mb, maize has a genome size of approximately 2,500 Mb, and wheat has a genome size of approximately 16,000 Mb, all of which are substantially larger than the approximately 100 Mb genome of *Arabidopsis*. In 2001, Monsanto also reported in a meeting that site-specific deletions in corn, wheat, soybean and cotton were generated using the Cre-lox system (Gilbertson *et al.*, Conference on In Vitro Technology St. Louis, In: *In vitro* Cellular & Development Biology Animal, March 2001 37(3 Part 2):26A, attached herewith). These references exemplify how those of ordinary skill in the art can readily extend the utility of a site-specific recombination system far beyond the initial demonstrations using model plant systems.

Moreover, Applicant asserts that transformation of cells with the constructs and vectors to perform the methods of the present invention are routine for one of ordinary skill in the art. Applicant notes that the quantity of experimentation needed to be performed is only one factor involved in determining whether undue experimentation is required to make and use the invention. "Time and difficulty of experiments are not determinative if they are merely routine."

MPEP §2164.06. Applicants submit that one of skill in the art would be able to make and use the claimed invention in a variety of cells types using the application as a guide. For example, the specification teaches that methods of introducing the constructs into a variety of cells may be accomplished using any technique known to those of skill in the art, including biolistic methods, electroporation, microinjection, PEG-mediated transformation, *Agrobacterium* mediated transformation, liposome-based DNA delivery, and viral vectors. Transformation and integration of DNA can be selected for using selectable markers as indicated in the instant specification. The integration patterns can be analyzed using methods well known in the art, including the analysis of DNA from the transformed cells, or the analysis of cells from an organism derived from the transformed cell, such as from a regenerated plant or its progeny, or from a transgenic or chimeric animal. These steps are all routine to one of ordinary skill in the art and do not constitute undue experimentation.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejections and allowance of Claims 1-16, and 35-36.

III. Rejections under 35 U.S.C. § 102

Claims 1-11, 13-16, and 35 were rejected under 35 U.S.C. § 102 as being anticipated by Groth *et al.*, PNAS USA, 97(11):5995-6000.

The Office Action asserts that Groth *et al.* teaches methods of obtaining site-specific gene replacement in a eukaryotic cell, comprising providing a eukaryotic cell that comprises a receptor construct wherein the receptor construct comprises a receptor polynucleotide flanked by two or more of an irreversible recombinase site, introducing into the cell a donor construct that comprises a donor polynucleotide flanked by two or more of an irreversible complementary recombination site, and contacting the receptor construct and the donor construct with a an irreversible recombinase site polypeptide, wherein the irreversible recombinase site catalyzes recombination between the first and second types of recombination sites and replacement of the receptor polynucleotide with the donor polynucleotide, thereby forming a replacement construct; where the cell is a mammalian cell, where the receptor construct is linear or circular vector, a chromosome, where the donor construct is a chromosome

and where the irreversible recombinase is a bacteriophage ϕC31 recombinase. Applicants respectfully traverse this rejection as follows.

Groth *et al.* discloses the integration and the excision of DNA using the bacteriophage ϕC31 recombinase in bacterial and in mammalian cells. In one experiment, a nucleic acid encoding LacZ is flanked by one copy of an attB site (an IRS), and one copy of an attP site (a CIRS). Upon exposure to the ϕC31 recombinase enzyme, the LacZ nucleic acid was excised if the attB and the attP sites were directly oriented, or the LacZ nucleic acid was inverted if the attB and attP sites were in inverted orientation.

In a second experiment, mammalian cells were transfected with two EBV-based plasmids. The experiment using the EBV-based plasmids was intended to mimic integration of DNA in a chromosome. One EBV-based plasmid contained an attB site, and the second EBV-based plasmid contained an attP site. Upon contacting the cell with the ϕC31 recombinase, integration occurred between the recombination sites on the two plasmids such that the recombined plasmid contained DNA from both of the original plasmids.

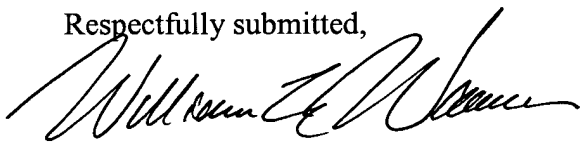
Groth *et al.* does not disclose a method for the replacement of a nucleic acid of interest. Groth *et al.* teaches a method for the integration of DNA at a recombination site through creating two separate constructs wherein the first construct comprises a first irreversible recombinase site and the second construct comprises an irreversible recombinase site that is complementary to the first recombinase site. Upon contact with an irreversible recombinase, integration of the two constructs will occur, however, this method does not allow for the replacement of DNA of one construct with the DNA from the second construct. Groth *et al.* further teaches flanking a nucleic acid of interest with one copy of an irreversible recombination site and one copy of a complementary irreversible recombination site. Upon contact with an irreversible recombinase, the nucleic acid of interest will be excised or inverted depending on the orientation of the recombination sites. Again, this method does not provide for the replacement of the nucleic acid of interest with a second nucleic acid.

To summarize, Groth *et al.* does not teach a method for obtaining site-specific replacement of a nucleic acid of interest; does not teach a receptor construct comprising a receptor polynucleotide flanked by two copies of an irreversible recombination site; does not

teach a donor construct comprising a donor polynucleotide flanked by two or more of an complementary irreversible recombination site, and does not teach replacement of the receptor polynucleotide with the donor polynucleotide, thereby forming a replacement construct. Therefore, Groth *et al.* cannot be said to anticipate the present claims, and the rejection should be withdrawn.

For at least the foregoing reasons, Applicants respectfully request reconsideration and removal of the rejections and allowance of Claims 1-16, and 35-36. The foregoing is submitted as a full and complete Response to the Final Office Action mailed May 7, 2003. No additional fees are believed due; however, the Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 19-5029. This Response places all claims in the present application in condition for allowance, and such action is courteously solicited. The Examiner is invited and encouraged to contact the undersigned attorney of record if such contact will facilitate an efficient examination and allowance of the application.

Respectfully submitted,



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